MATERNAL SERUM ALPHA FETO PROTEIN SCREENING IN HIGH RISK PATIENTS AND PREGNANCY OUTCOME

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled "**MATERNAL SERUM ALPHA FETO PROTEIN SCREENING IN HIGH RISK PATIENTS AND PREGNANCY OUTCOME**" is a bonafide work of **Dr. D. ANGAMMAL**, **Roll Number – 20031552**, who carried out the dissertation under my supervision. Certified further that to the best of my knowledge, the work reported herein does not form part of any other thesis or dissertation on the basis of which a degree or award was conferred on an earlier occasion on this or any other candidate.

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DECLARATION

I, **Dr. D. ANGAMMAL** solemnly declare that this dissertation titled "**MATERNAL SERUM ALPHA FETO PROTEIN SCREENING IN HIGH RISK PATIENTS AND PREGNANCY OUTCOME**" is a bonafide work done by me at Government RSRM lying in hospital Stanley Medical College, during the period of December 2004 to January 2006 under the guidance and supervision of **Prof. DR. S. DEVAMBIGAI,** M.D., D.G.O, HOD & Superintendent of Govt. R.S.R.M Lying In Hospital, Stanley Medical College, Chennai – 600 013.

This dissertation is submitted to The Tamilnadu Dr. MGR Medical University towards partial fulfillment of requirement for the award of MD Branch II obstetrics and gynaecology.

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Date :

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Introduction

Congenital abnormalities have a major impact on neonatal morbidity / mortality as well as a heavy emotional burden on the family. To identify them prenatally is an essential task of the obstetrician, who is involved in the care of the pregnant women.

Prenatal diagnosis is the art and science of identifying structural and functional abnormalities which includes screening methods and definitive diagnostic procedures. Screening identifies individuals whose risk is high enough that they could benefit from further evaluation. Screening methods include assessment of serum markers like AFP, hCG, UE_3, inhibin A, PAPP-A, and USG assessment of congenital anomalies.

Definitive diagnostic procedures include amniocentesis, CVS, fetal blood sampling, and Preimplantation genetic diagnosis which allows analysis of embryonal and fetal cells or tissues for chromosomal, genetic and biochemical abnormalities.

Maternal serum alpha feto protein is a simple and cost effective screening method. Though initially discovered to identify neural tube defects three decades ago, studies have documented that the values of MSAFP

estimation extends well beyond the detection of NTD in the fetus [Thomas and Karim 1990].Abnormally elevated and low levels of MSAFP are an indication of high risk pregnancy and sub optimal outcome of the pregnancy [Burton 1998, Burton et al 1983, 1986].

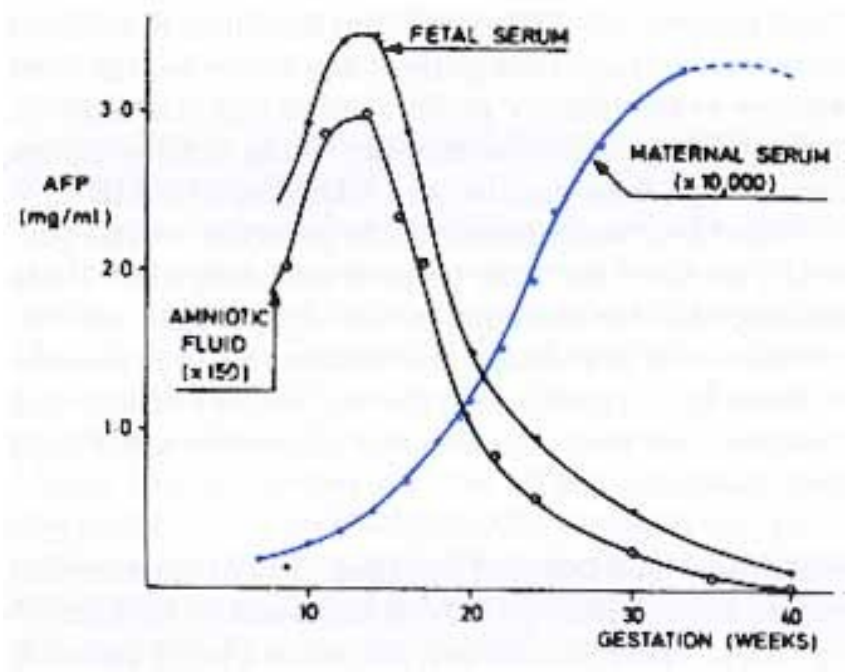
Before screening, the patients should receive counseling which includes the purpose of the tests, the risks involved, limitations of the screening tests and the patient's options.

The implications of early detection of abnormalities include

- Emotional preparation of the couple
- Elective termination of the affected fetus
- Enable clinician to provide more intense obstetric care and optimize labour and delivery of the affected patients.
- To reduce the birth prevalence of severely affected fetuses.

Review of Literature

- In 1956- AFP was discovered by Bergstrand and Czar. It is the first major protein component to appear in fetal serum. AFP is a glycoprotein similar to albumin in structure and molecular weight. It is first made in the fetal yolk sack at four to eight weeks. As the yolk sac degenerates by eleven weeks, fetal liver takes over the function of producing the AFP. Genes encoding AFP are in the long arm of chromosome 4. Function of AFP is unknown although an immune / carrier function has been suggested. Fetal serum AFP increases in first trimester rising from the sixth week, reaches a peak value of 3mg/ml at thirteen weeks. Production of AFP by fetal liver continues to increase up to twenty weeks and there after remain constant up to thirty two weeks due to the disproportionate increase in fetal growth. After thirty two weeks, production falls sharply and fetal serum AFP falls rapidly [GETLIN and BORMAN 1960]. Fetal serum AFP enters amniotic fluid largely via fetal urination after renal infiltration. AFAFP rises until approximately 12 weeks and there after declines. AFP diffuses across the placenta [2/3] and amnion [1/3] to reach the maternal serum. Amniotic fluid to maternal serum level ranges from 100: 1 to 200:1. MSAFP value ranges from mg/ml in fetal serum to µgm/ ml in amniotic fluid to ng/ml in maternal serum.



AFP in Normal Pregnancy

- Origin and regulation of AFP depends on the rate of synthesis , catabolism of AFP by the fetus, changes in permeability of feto maternal barrier, volume of body fluid , disturbance in circulation of body fluids and feto maternal transfusion [Sappala 1973, Ishguno 1973, Wald et al 1975, Brock 1976, Getlin 1975].

- Open neural tube defects have a thin covering or only a thin membrane over the brain / spinal cord, so AFP can leak to the surrounding amniotic fluid and secondarily in to the maternal serum.

- In 1972 Brock and Sutcliffe showed that AFAFP are higher in pregnancies with fetal anencephaly.

- In 1973 Brock and Associates showed that MSAFP are also elevated in affected pregnancies.

- 10 to 15% of spinal NTD, 1 % of anencephaly, 50% of encephalocele are skin covered and do not leak AFP and hence are not associated with increased MSAFP.

- In 1977 First UK collaborative study developed the mathematical principles of screening and presented a rational system upon which screening policy decisions are based. It also defined the optimal time of gestation for screening [16 – 18 weeks] and then the boundaries beyond which screening is either ineffective [< 15 weeks] or not feasible [> 22 weeks].

- In 1984, Merkatze et al showed that MSAFP averaged lower in pregnancies affected by Down syndrome. It was confirmed by other studies- Clarke and colleagues [1984], Haddow and associates [1983].

- In 1986-1987 a multi center intervention trial of combining maternal age and MSAFP to screen for Down syndrome in women < 35 years in the state of Lowa, showed a detection rate of 25% for Down syndrome.

- In 1987 Bogart and colleagues and in 1988 Wald and associates showed that hCG and estriol can also be used for screening for Down syndrome.

- In 1988 a large case controlled study which simultaneously examined AFP, uE_3 and hCG and determined that the three bio chemical markers are independent of maternal age and largely independent of each other.
- Thus MSAFP screening became the framework on which other pregnancy screening tests were added.
- Several large studies have documented increased perinatal morbidity and mortality including LBW, Oligohydramnios, preterm birth and fetal death in patients with unexplained elevated MSAFP. Some of them are

- Simpson and colleagues [1991] in their study reported that there was a significant association between second trimester elevated AFP and PPROM, preterm labour and LBW.

- Ramus and associates [1996] reported an increased incidence of preterm delivery in women with unexplained serum AFP elevation.

- In 1994 Reicheler and colleagues reported that there was a progressive increase in frequency of NTD, ventral wall defects and fetal anomalies as a direct function of maternal serum level.

- In 1996 ACOG recommends that all pregnant women be offered second trimester MSAFP screening as 90 to 95 % of infants with NTD are born to families with no history of NTD and 85% of Down syndrome is born to women < 35 years.

Laboratory Aspects of MSAFP

1. Normative data should be established for the population served by the laboratory.
2. Assay precision and accuracy must be monitored.
3. Precision refers to the coefficient of variation when performing replicate tests on the same samples.
4. Accuracy refers to the laboratory result compared to a known external standard.
5. Factors influencing MSAFP levels should be corrected.

Factors Influencing MSAFP values

- Gestational age: MSAFP steadily increases about 15% / week during the second trimester. MSAFP continues to rise (in contrast to fetal AFP) until 30 to 32 weeks before declining.
- Race: MSAFP is 10 to 15% higher in black women.
- Maternal weight: MSAFP value is lower in heavier woman because of dilution effect.
- Multiple gestation: Levels increase due to increased number of fetuses and the cut off for elevated value is twice than normal.
- IUD: Levels increase due to disruption of placental barrier.
- IDDM: IDDM is associated with MSAFP that averages 40% lower than general pregnant population.

MSAFP ESTIMATION

Principles of the Assay

The essential reagents required for an immunoenzymometric assay include high affinity and specific antibodies, with different and distinct epitope recognition, in excess and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti –AFP antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen reaction results

between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Materials Provided in the Kit

1. Alpha fetoprotein(AFP) - 1 ml/vial
    - Six vials of references AFP antigen at levels of 0, 5, 25, 50, 250 and 500 ng /ml.
2. Anti-AFP Enzyme reagent - 13 ml/vial
    - One vial containing enzyme labeled antibody, biotinylated monoclonal IgG
3. Streptavidin Coated microplate - 96 wells.
    - One 96 – well microplate coated with streptavidin
4. Wash solution concentrate - 20 ml
    - One vial containing a surfactant in buffer.
5. Substrate A - 7 ml/vial
    - One bottle containing tetra methyl benzidine in buffer.
6. Substrate B - 7ml/vial
    - One bottle containing hydrogen peroxide in buffer.
7. Stop solution - 8 ml/vial
    - One bottle containing a strong acid (1N HCl).

Storage and Stability

- The kits should be stored at 2 to 8^{o}C and micro well is kept in a dry bag with desiccants.
- Solution A and B should be colourless: If the solution turns blue it must be replaced. These reagents should not be exposed to strong light during storage or usage.

Specimen Collection and Handling

The blood is collected by venipuncture, allowed to clot and the serum is separated by centrifugation at room temperature. If sera cannot be assayed immediately, they can be stored at 2 to 8^{o}C or frozen.

Preparation for Assay

- All reagents and samples are brought to room temperature (20 to 25^{o}C) and gently shaken before beginning the test.
- All reagents and samples are kept ready before the start of the assay. Once the test is started it must be performed without any interruption to get the most reliable and consistent results.
- New disposable tips are used for each specimen.

Assay Procedure

1. The microplate wells for each serum reference, control and patient specimen to be assayed in duplicate are formatted.
2. 25 µl of the appropriate serum reference, control or specimen is pipetted out into the well.
3. 100 µl of the anti –AFP Enzyme reagent is added to each well.
4. The microplate is swirled gently for 20—30 seconds.
5. Incubation is done for 60 min at room temperature.
6. The contents of the microplate are discarded by decantation or aspiration.
7. 300 µl of wash buffer is added and it is used in an automatic washer and a total of three washes given.
8. 100 µl of the working substrate solution is added to all wells.
9. Incubation is done at room temperature for 15 minutes.
10. 50 µl of stop solution is added to each well and mixed gently for 15 to 20 seconds.
11. The absorbance in each well is read at 450 nm in a microplate reader.

Calculation of results

1. The concentration (X) of each reference standard is plotted against its absorbance (Y) on full log arithmic graph paper
2. AFP value of the patient is obtained by referring to the standard curve

MSAFP is measured in ng/ml. Normal non pregnant adult serum AFP is < 10 ng/ml. In pregnant women MSAFP value increases with advancing gestation to approximately 30 weeks, then plateaus until 36 weeks and then falls.

MSAFP value in pregnancy is expressed as MOM of the unaffected population. MOM is obtained by dividing an individual's MSAFP value by the median for the relevant gestational week.

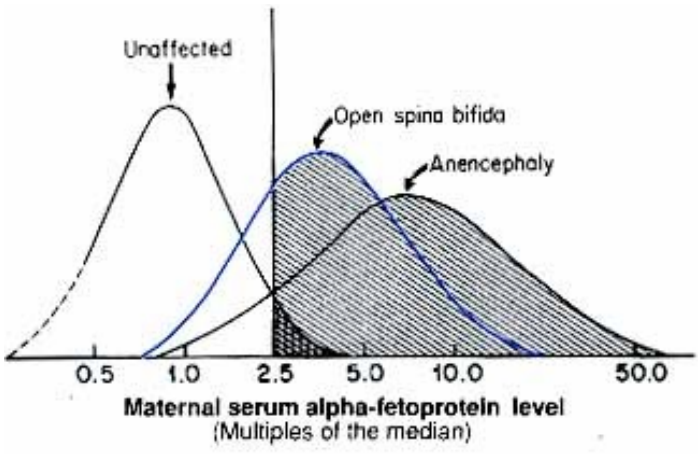
Using the MOMs to normalize AFP values

1. Allows information from different laboratories to be interpreted in a common manner.
2. Normalizes rising MSAFP and reducing AFAFP during the period of screening.
3. Can be readily adjusted for variables which affect AFP such as diabetes, weight and race.

4. It also provides a more precise parameter for setting screening cut off as opposed to standard deviation and percentile rate.

Normal range of MSAFP Value	0.5 to 2.5 MOM
Cut off values for Elevated MSAFP value	≥ 2 to 2.5 MOM
Cut off values for Low MSAFP value	≤ 0.5 MOM
Cut off values in Multiple pregnancy	≥ 4.5 to 5.0 MOM (twice than normal)
Cut off values in IDDM	≥ 1.5 MOM (40 % lower than general population)

Distributions of serum AFP overlap in affected and unaffected pregnancies. If the level is within the range of overlap, the indiscriminate zone of 2.5 to 3.5 MOM, then repeating measurement may determine to which distribution the sample actually belongs – affected / unaffected.



The cut off value of 2.5 multiples of the median results in both false – positive (cross hatched area) and false negative diagnosis.

If MSAFP is ≥ 3.5 MOM then measurement need not be repeated as levels this high are outside AFP distribution of unaffected pregnancies and associated with increased fetal risk.

Follow Up of Patients with Abnormal MSAFP

Conditions associated with elevated AFP levels

1. Neural tube defects
2. Pilonidal cysts
3. Esophageal or intestinal obstructions
4. Liver necrosis
5. Cystic hygroma
6. Sacrococcygeal teratoma
7. Abdominal wall defects – omphalocele, gastroschisis
8. Urinary obstructions
9. Renal anomalies – Polycystic or absent kidney
10. Congenital nephrosis
11. Osteogenesis imperfecta
12. Congenital skin defects
13. Cloacal exstrophy
14. Amniotic band disruption
15. Fetal growth restriction and death
16. Fetomaternal haemorrhage

17. Preterm membrane rupture and delivery
18. Chorioangioma
19. Low birth weight
20. Oligohydramnios
21. Multifetal gestation
22. Decreased maternal weight
23. Underestimated gestational age
24. Maternal hepatoma or teratoma, acute viral hepatitis, lupus antibody.

Conditions associated with low AFP levels

1. Chromosomal trisomies
2. Gestational trophoblastic disease
3. Fetal death
4. Increased maternal weight
5. Overestimated gestational age

Using a MSAFP level of 2 to 2.5 as the upper limit of normal, most laboratories report

- Screen positive rate of 3 to 5 %
- Sensitivity of 90%

- Positive predictive value of 2 to 6% (Milunsky and associates 1989)

But the main problem is that MSAFP testing has a high rate of false positives. Only a small fraction of those patients with abnormally high or low MSAFP values will actually have affected babies.

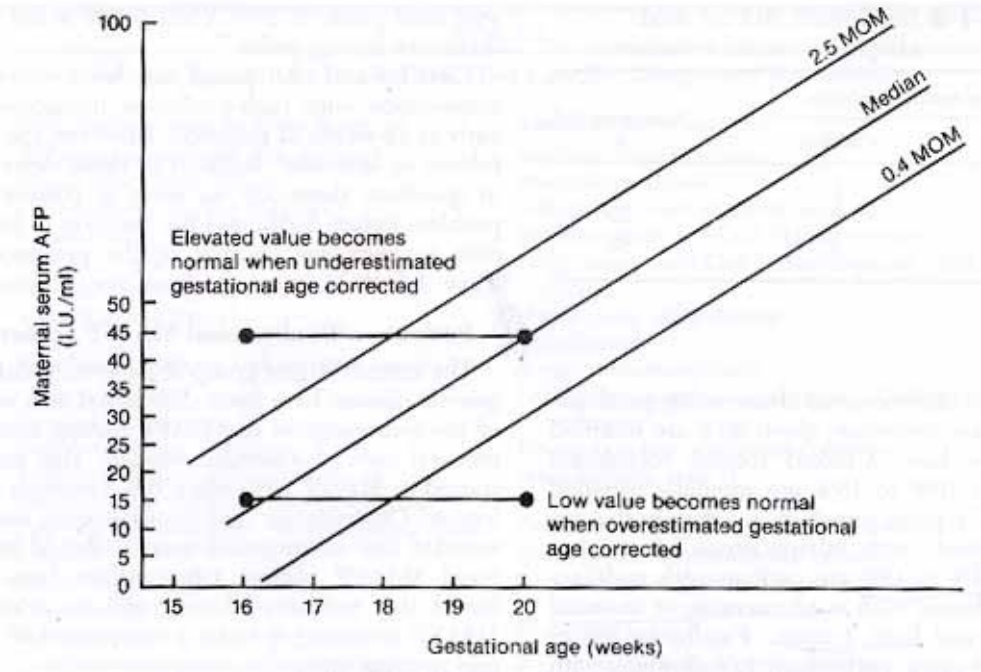
Burton et al found only 15 out of 452 women with abnormal MSAFP actually had affected fetus.

Simpson et al found only 1 out of 187 women with abnormal MSAFP had an affected fetus.

Therefore it is necessary to follow a series of steps in the analysis of patients with increased or decreased MSAFP values to avoid an in correct diagnosis.

Recommendations for follow up of patients with elevated MSAFP

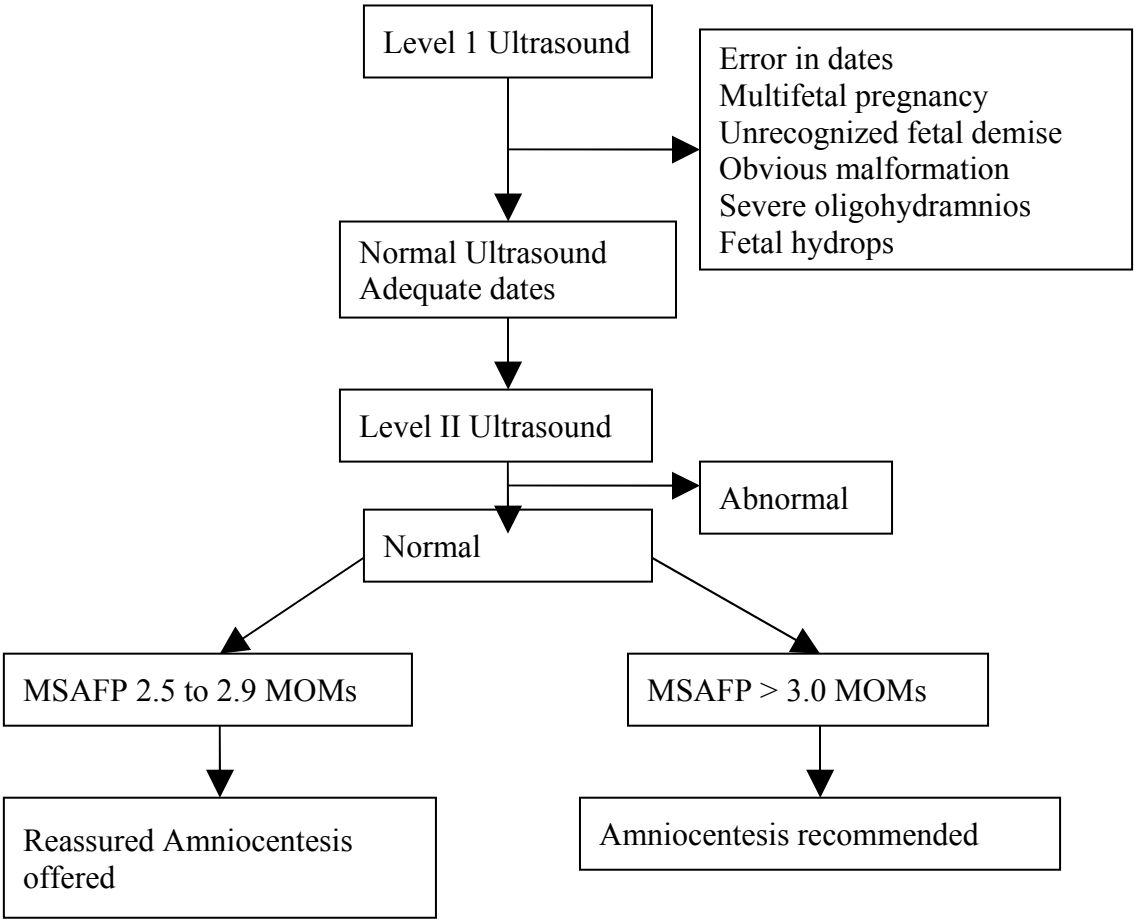
MSAFP values above 2.5MOM are considered abnormal. When a patient has an elevated MSAFP level, an USG (Level—1) should be performed. USG can detect most common non genetic reasons causing high MSAFP values. An elevated MSAFP may result from an error in the dating of the pregnancy. A high value may become normal when an underestimated gestational age is corrected .20 to 25% of patients with elevated MSAFP, when reassigned to correct GA, will not need further testing.



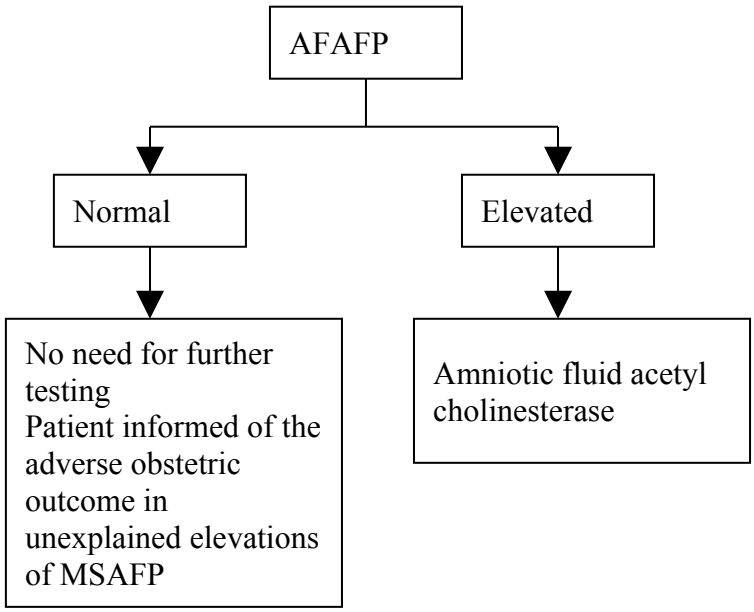
Other conditions associated with elevated MSAFP which can be diagnosed by an USG (Level-1) include

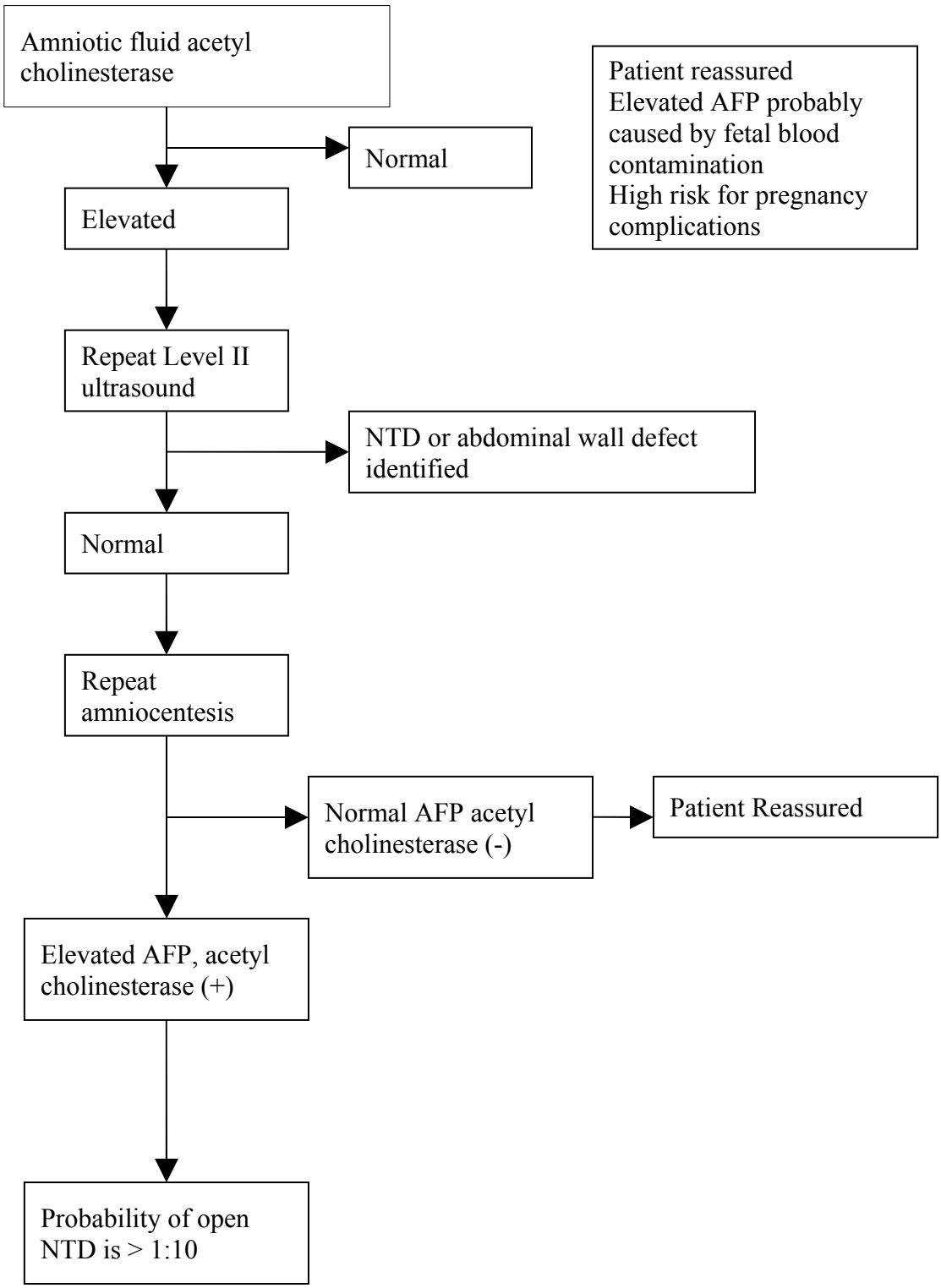
- Multiple gestation in 15% of cases
- Undiagnosed fetal demise in 5% of cases
- Severe oligohydramnios
- Obvious anatomical defects in 2 to 3% of cases
- Other rare conditions like fetal hydrops which cause elevated MSAFP

After a level I USG has ruled out the above conditions, a level II USG is done. If it is normal, then further management depends on the level of elevated MSAFP Amniocentesis is definitely recommended if level is > 3 MOM.


Level 1 Ultrasound
Error in dates
Multifetal pregnancy
Unrecognized fetal demise
Obvious malformation
Severe oligohydramnios
Fetal hydrops
Normal Ultrasound
Adequate dates
Level II Ultrasound
Abnormal
Normal
MSAFP 2.5 to 2.9 MOMs
MSAFP > 3.0 MOMs
Reassured Amniocentesis offered
Amniocentesis recommended

The objective of genetic amniocentesis in patients with elevated MSAFP is to determine the concentration of AFP in the amniotic fluid .AFAFP determination is a diagnostic test, in contrast to MSAFP which is a screening test.




Amniotic fluid acetyl cholinesterase
Normal
Patient reassured
Elevated AFP probably caused by fetal blood contamination
High risk for pregnancy complications
Elevated
Repeat Level II ultrasound
NTD or abdominal wall defect identified
Normal
Repeat amniocentesis
Normal AFP acetyl cholinesterase (-)
Patient Reassured
Elevated AFP, acetyl cholinesterase (+)
Probability of open NTD is > 1:10

In most centers, chromosome analyses are performed on all amniotic fluids obtained because of elevated MSAFP levels. When AFAFP level is elevated, chromosome analysis is of value, since a significant proportion of fetuses with NTD also have chromosomal abnormalities. Also knowledge of the karyotype may be important for parents considering termination of pregnancy.

Unexplained elevations of MSAFP

Elevated MSAFP with normal USG and normal level of AFAFP will forecast a poor pregnancy outcome, even with no obvious fetal anomalies. Increased perinatal morbidity and mortality including LBW, oligohydramnios, placental abruption, preterm birth and fetal death are noted.

The placenta either secondary to providing increased areas of transport or in providing an abnormal endothelial barrier provides for greater transfer of fetal serum AFP and thus AFAFP in to the maternal compartment. An abnormal placenta is also a likely explanation for the increased risk of adverse pregnancy outcome that is associated with increased MSAFP for which no etiology is found

Therefore knowledge of increased MSAFP unassociated with fetal structural malformations should trigger a modification of prenatal care to provide enhanced fetal and maternal surveillance

Recommendations for Patients with low MSAFP levels

After correcting for the low MSAFP value for weight, presence of IDDM and the race to which the patient belongs, an USG is done to correct GA and rule out missed abortion /blighted ova.

If still the MSAFP value is found to be less than 0.25 MOM, genetic amniocentesis is done for karyotyping, as MSAFP on average is 25% less in trisomies than the level in women with chromosomally normal fetus and is not dependent on age. Down syndrome risk increases steadily but slowly in relation to a woman's age until mid thirties after which the risk increases at an accelerated pace. AFP levels in affected/ unaffected population are independent of her age, hence both can be combined to derive a risk and increase the overall screening efficiency rate.

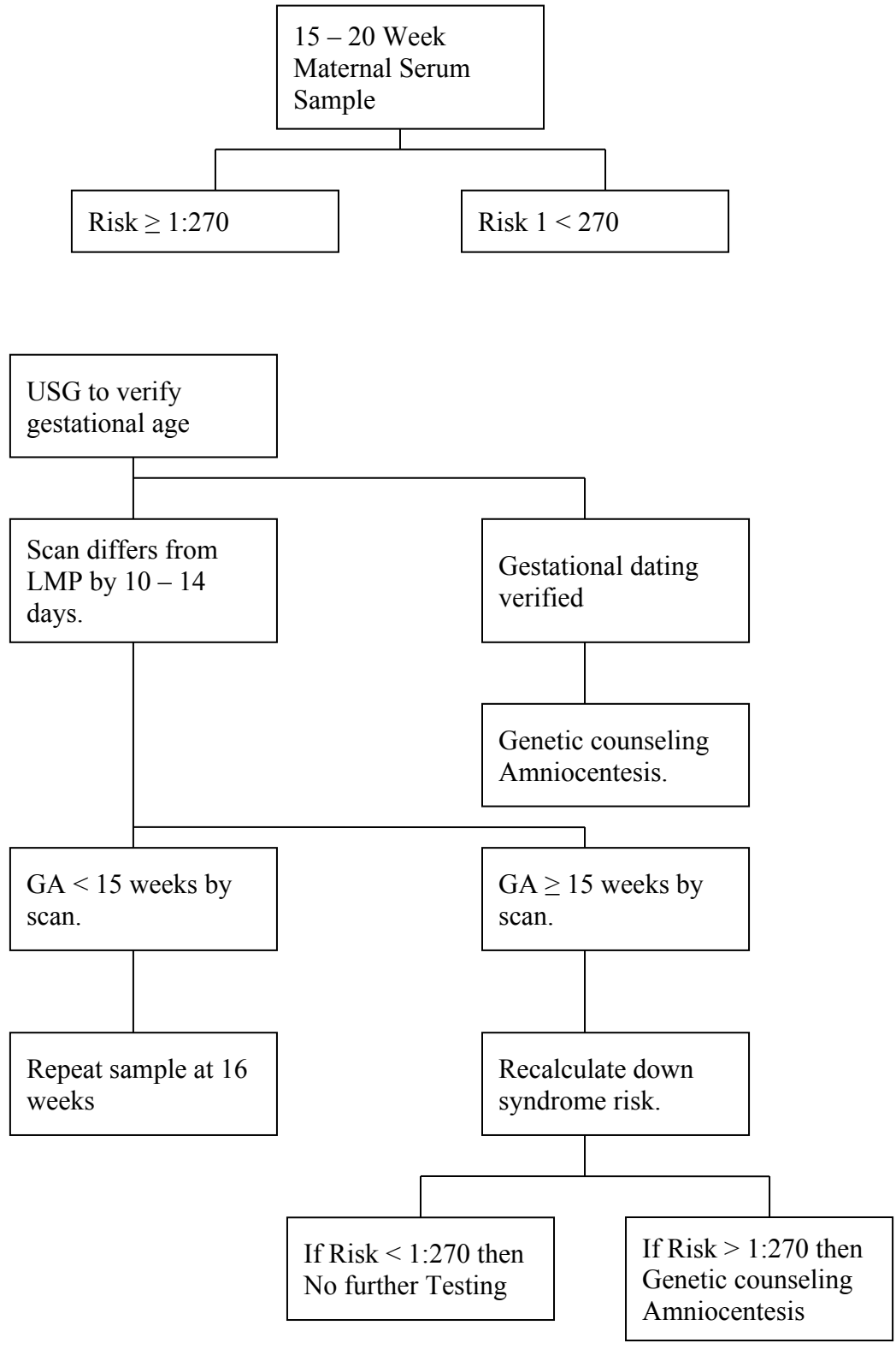
When maternal age alone is used a screening factor 31% of Down syndrome is detected , with a false positive rate of 7.5% .When MSAFP estimation is done in women <35 years, detection rate for Down syndrome is 25 – 30% .

Specific fetal aneuploid conditions commonly detected through maternal serum analyte screening include Down and Edward syndrome. Evidence indicates that the measurement of AFP in maternal serum can discriminate chromosomal anomalies as early as 9 to 11 weeks gestation.

MSAFP screening became the frame work upon which other pregnancy screening tests were added. It was discovered that hCG in maternal serum to average more than 2 times higher in Down syndrome and uE_3 was reported to average 25% lower in Down syndrome .Combining all the 3 tests, 80% of all autosomal trisomies are detected. When all three markers are combined to screen, it is called triple test .Recently dimeric inhibin A is added to the list and it is called Quad screen.

When karyotyping is normal in the presence of low MSAFP there is 30% probability of an abnormal outcome of the pregnancy like spontaneous abortion, fetal death, hydatiform mole and choriocarcinoma.

Screening Protocol for patients with Low MSAFP



Aim of the study

To evaluate the usefulness of Maternal Serum Alpha Feto Protein estimation as a screening method to identify the mothers at risk of

- Neural tube defects
- Other congenital anomalies
- Chromosomal abnormalities
- Other adverse pregnancy outcomes such as LBW, IUGR, spontaneous abortion, preterm birth, still birth and neonatal complications, so that effective and timely therapeutic measures can be taken to modify the fetal outcome

Materials and Methods

Study is carried out in Government RSRM hospital, Stanley Medical College, during the period from December 2004 – January 2006

Study group comprised of 75 cases of pregnant patients at GA 15 to 22 weeks who attended our antenatal OPD with any one of the following high risk factors

1. Age above 35 years
2. Previous H/O early pregnancy loss
3. Previous H/O congenital anomalies
4. Previous H/O neural tube defects
5. Previous H/O baby with Down Syndrome
6. Family H/O congenital anomalies/ chromosomal disorder
7. Known epileptic patient on treatment
8. Anemia complicating pregnancy
9. Fetuses exposed to any teratogen.

Most of the patients had regular menstrual cycles, were not on any oral contraceptives and they knew their LMP correctly. For patients with irregular cycles/ unreliable dates, gestational age was determined by a dating scan.

A detailed work up of each patient was carried out according to well designed proforma. A detailed history was taken and a thorough physical examination was performed. Routine investigations included Hb, urine analysis, Blood Grouping/ Typing and VDRL.

For the subjects in the study group blood 3cc was collected by the venipuncture in a sterile test tube and sent to the laboratory where MSAFP measurement was done. The blood was allowed to clot and the serum was separated by centrifugation at room temperature and stored in -20ºC deep freezer.

Method

Test is a solid phase enzyme linked immunosorbent assay. This test provides quantitative measurement of human alpha feto protein in serum and in amniotic fluid. Detailed principles of the assay of serum AFP is explained before in the section of laboratory aspects of MSAFP.

Calculation of Results

1. The concentration of each reference standard is plotted against its absorbance on full logarithmic graph paper
2. AFP value of the patient is obtained by referring to the standard curve

The results obtained were compared with the standard value (in MOM) of the laboratory. Those patients whose MSAFP concentration was above 2.5 MOM underwent USG. If anomalies were found, after counseling, the pregnancy was terminated. In case where there were no ultrasonologically detectable anomalies, cases were followed up till the termination of pregnancy with special reference to fetal weight, gestational age, apgar score and perinatal outcome.

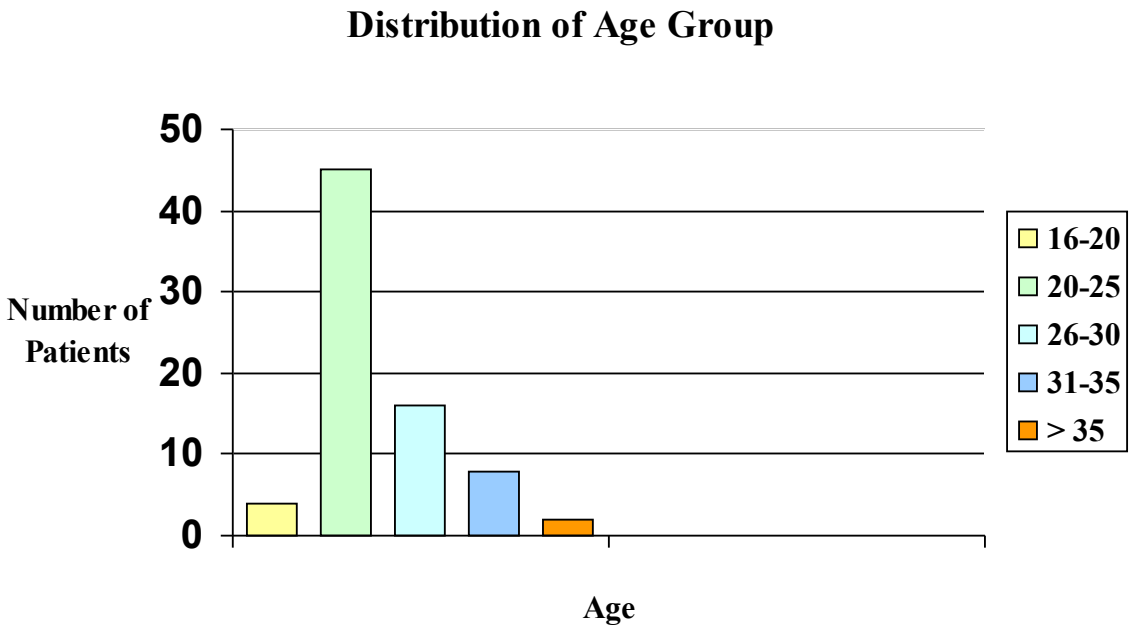
Results

The study group consisted of 75 patients at GA 15 to 22 weeks who had any of the risk factors cited above. MSAFP screening was done for these patients and the value of MSAFP was converted from ng/ml to MOM by dividing the patient's value with the mean value for the particular GA.

Values above 2.5 MOM were considered elevated and < 0.5 MOM were considered low. All patients were followed till delivery and the pregnancy outcome was noted The relation between abnormal MSAFP value and adverse pregnancy outcome was correlated.

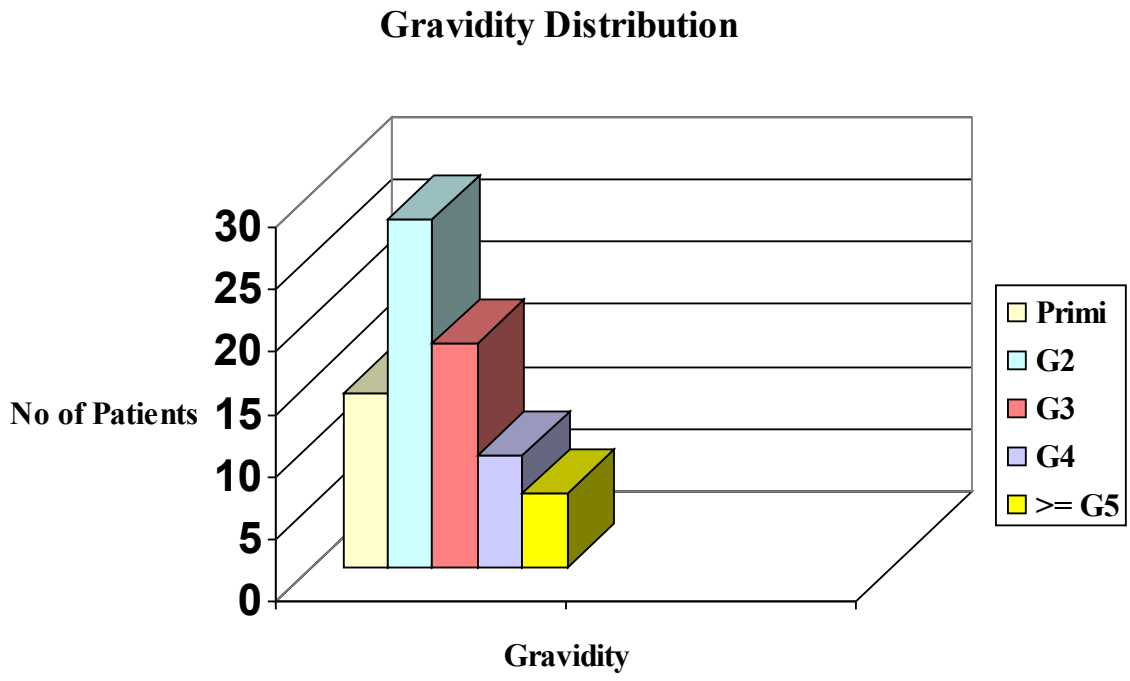
Distribution of Age Group

Age	Number of patients	Percentage
16-20	4	5.33%
20-25	45	60%
26-30	16	21.33%
31-35	8	10.66%
> 35	2	2.66%
Observation	60 % of the patients lie in the 20-25 age group	



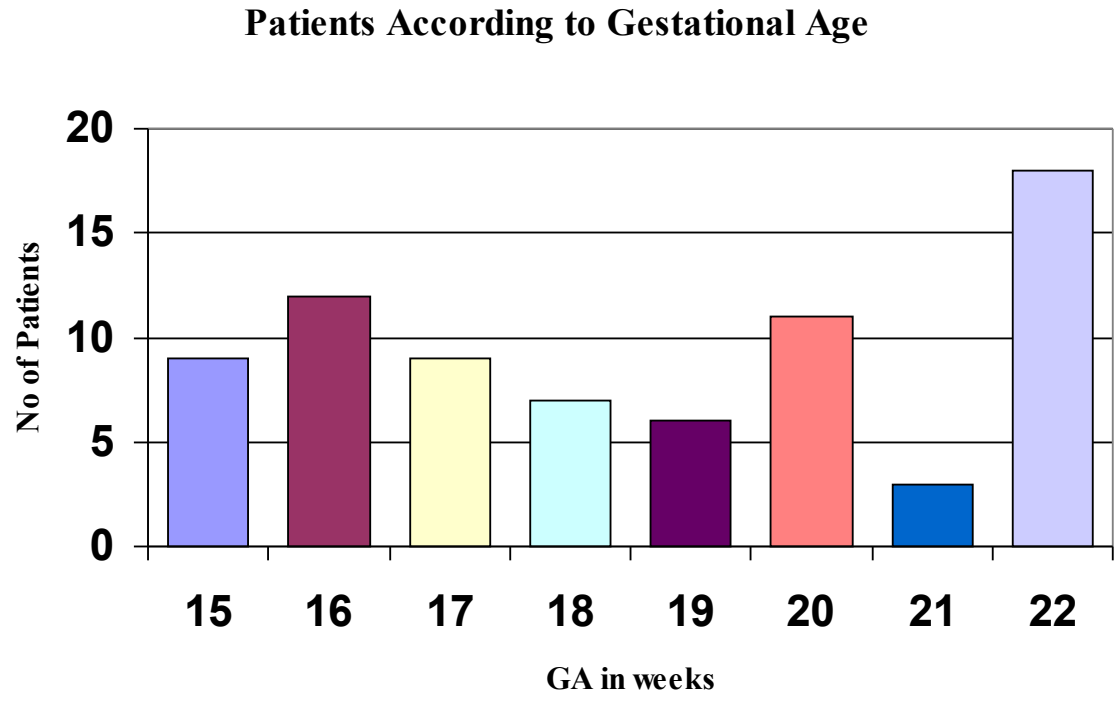
Distribution of Gravidity

Gravidity	Number	Percentage
Primi	14	18.66%
G2	28	37.33%
G3	18	24%
G4	9	12%
G5	6	8%
Observation	Most of the patients are second gravida	



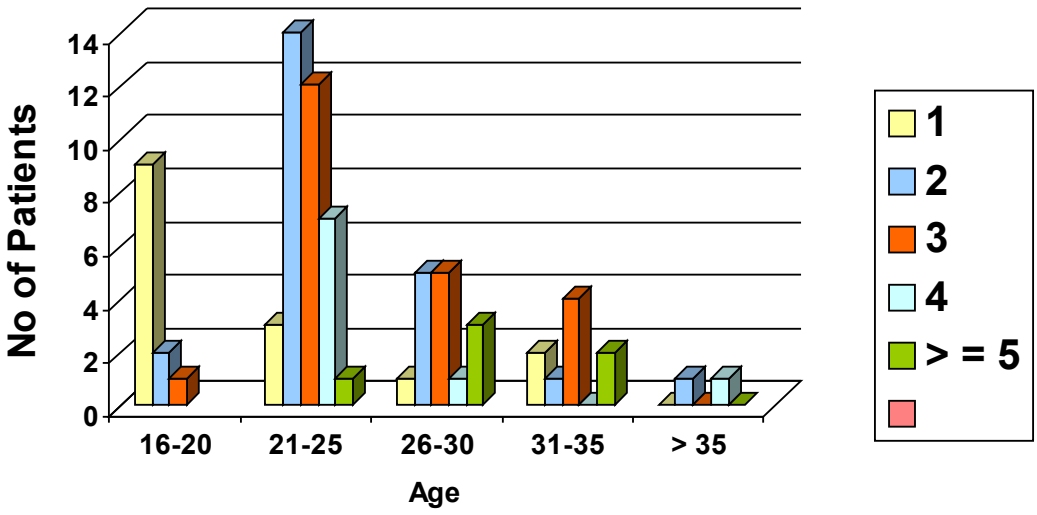
Distribution of Patients according to Gestational Age (GA)

GA	Number	Percentage
15	9	12%
16	12	16%
17	9	12%
18	7	9.33%
19	6	8%
20	11	14.66%
21	3	4%
22	18	24%
Observation	Most of the patients screened were at 22 weeks, though the screening was done between 15 to 22 weeks	



Gravidity Distribution According to Age

Gravidity	Age				
	16-20	21-25	26-30	31-35	>35
1	9	3	1	2	0
2	2	14	5	1	1
3	1	12	5	4	0
4	0	7	1	0	1
>=5	0	1	3	2	0



Laboratory Standard Value of MSAFP for Each Week of Gestation in MOM

GA(weeks)	Median value of MSAFP	Multiple of Median	
		2.5	0.5
15	11	27.5	5.5
16	14	35	7
17	20	50	10
18	27	67.5	13.5
19	35	87.5	17.5
20	42	105	21
21	50	125	25
22	60	150	30
	This table shows median according to gestational age. This standardized mean was followed in the study.		

No of cases showing elevated MSAFP level according to gestational age

Total Number of Cases – 75

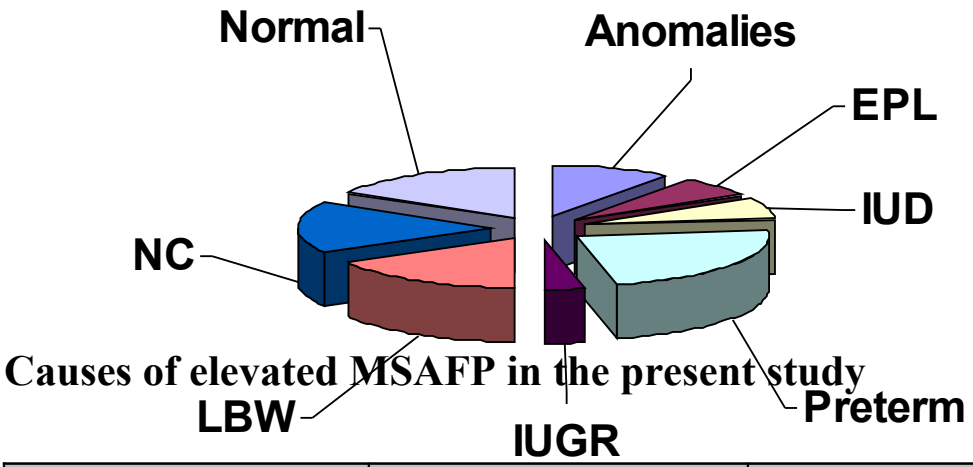
GA	Total Number of Patients	Number of Patients with Elevated MSAFP	Percentage
15	9	5	55.55%
16	12	4	33.33%
17	9	2	22.22%
18	7	4	57.14%
19	6	3	50%
20	11	5	45.45%
21	3	0	0
22	18	5	27.77%
Observation	Out of the study group 37.33% showed elevated MSAFP value. 57.14% in the 18 weeks gestation showed elevated MSAFP level.		

Patients according to high risk factor

Total Number of Cases – 75

High Risk Factors	Number	Percentage
BOH	26	34.66%
Hypertension / BOH	5	6.66%
Known epileptic / BOH	2	2.66%
BOH/ Anemia	1	1.33%
Hypertension complication pregnancy	3	4%
Positive family history of Hypertension	2	2.66%
Anemia / Hypertension	2	2.66%
Anemia	6	8%
Anemia / previous history of anomalous baby	1	1.33%
Anemia / Positive family H/O	1	1.33%
Known epileptic on treatment	3	4%
Known epileptic on treatment / positive family history	1	1.33%
Fever	3	4%
Previous history of anomalous baby	6	8%
Drug intake	5	6.66%
Positive family history	6	8%
Elderly Gravida	2	2.66%
Observation	Maximum number of cases screened were with history of BOH- 34.66%	

Causes of Elevated MSAFP



Causes	Number of Cases	Percentage
Anomalies	3	10.71%
Early pregnancy loss	2	7.14%
IUD	2	7.14%
Preterm	7	25%
IUGR	1	3.57%
LBW	5	17.85%
Neonatal complications	3	10.71%
Normal	5	17.85%

Type of congenital anomalies in elevated MSAFP

Total no of congenital anomalies - 3

CNS anomalies - 2

Other anomalies - 1

S.No	Anomaly	Number
1	Anencephaly	1
2	Spinabifida	1
3	Exomphalos	1

Association of elevated MSAFP Level and Congenital Anomalies

Diagnosis	MSAFP		
	Normal	Elevated (≥ 2.5 MOM)	Low (≤ 0.5 MOM)
Congenital Anomalies	Nil	3	Nil
Observation	1. MSAFP levels are elevated in all the 3 cases of pregnancies with anomalous babies.		

- Among the study group 3 (4%) had congenital anomaly.
- Among the congenital anomalies 100% had elevated MSAFP
- 10.71% of patients with elevated MSAFP had congenital anomaly.

Association of MSAFP Level and preterm deliveries

Diagnosis	Number of cases	MSAFP		
		Normal	Elevated (2.5 MOM)	Low (0.5 MOM)
Preterm deliveries	8	1	7	Nil
Observation	1. Among study group 8(10.66%) went into preterm labour. 2. Out of 8 cases 7 had elevated MSAFP (87.5%) 3. 1 patient had normal MSAFP (12.5%) 4. 25% of patients with elevated MSAFP level had preterm deliveries.			

Association of MSAFP level and IUGR

Diagnosis	Number of cases	MSAFP		
		Normal	Elevated (2.5 MOM)	Low (0.5 MOM)
IUGR	1	Nil	1	Nil
Observation	1. Among the study group 1(1.33%) had IUGR. 2. Hence 100% of patients with IUGR had elevated MSAFP. 3. 3.75% of patients with elevated MSAFP had IUGR.			

Association of MSAFP Level and LBW

Diagnosis	Number of cases	MSAFP		
		Normal	Elevated (2.5 MOM)	Low (0.5 MOM)

LBW excluding preterm	5	2	3	Nil
Observation	1. Among the study group 5(6.66%) had LBW. 2. Among the LBW 60% had elevated MSAFP. 3. 40% had normal MSAFP. 4. 10.71% of patients with elevated MSAFP had LBW.			

Association of early pregnancy loss and MSAFP

Diagnosis	**Number of cases**	**MSAFP**		
		Normal	**Elevated (2.5 MOM)**	**Low (0.5 MOM)**
Incomplete abortion	2	1	1	Nil
Complete abortion	1	Nil	1	Nil
Observation	1. Among the study group 3 (4%) had early pregnancy loss 2. 66.66% had elevated MSAFP 3. 33.33% had normal MSAFP 4. 7.14% of patients with elevated MSAFP had early pregnancy loss			

Fetal outcome in the study group (75 Patients)

Diagnosis	Number of cases		MSAFP Level					
			Normal		Elevated		Low	
	No	Percentage	No	%	No	%	No	%
Still Birth	2	2.66%	-	-	-	-	-	-
Fresh	1	1.33%	-	-	1	100	-	-
Macerated	1	1.33%	-	-	1	100	-	-
Neo natal death	1	1.33 %	1	100	-	-	-	-
Anomalies	3	4%	-	-	3	100	-	-
CNS	2	2.66%	-	-	2	100	-	-
Omphalocele	1	1.33%	-	-	1	100	-	-
Neonatal complications	3	4%	-	-	3	100	-	-

This study shows 2.66% of still births in the study group .All of them showed elevated MSAFP levels.

One of the still births is a macerated IUD, delivered at 7 months by a mother who had previous four abortions.

The other still birth is a fresh IUD delivered at 6 months, by a mother who was a case of severe PIH.

One neonatal death occurred but the mother had normal MSAFP value. The baby died of respiratory distress 2 days after delivery. Autopsy was not done as the parents were not willing to subject the baby for autopsy.

4% of the study group had anomalous babies out of which 66.66% had CNS anomalies

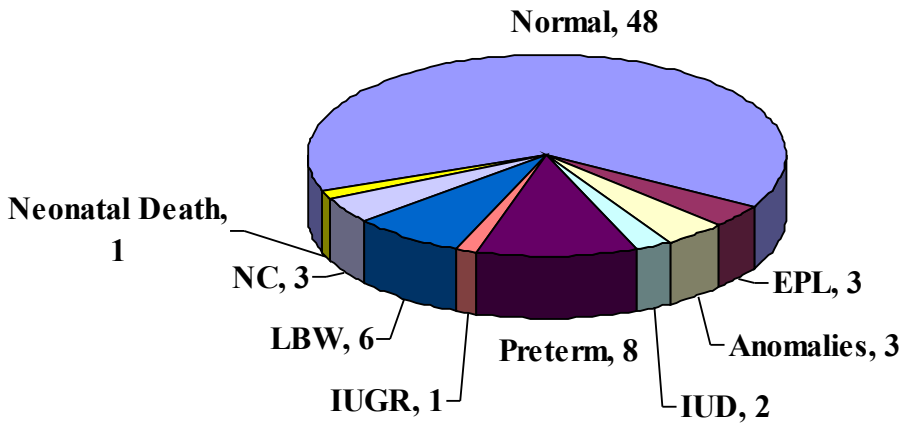
(anencephaly and spinabifida) and 33.33% had ventral wall defect (exomphalos).

Among the study group 4% had neonatal complications and all had elevated MSAFP

Pregnancy outcome in the study group of 75 patients

Outcome	Number	Percentage
Early Pregnancy Loss	3	4%
Congenital Anomalies	3	4%
IUD	2	2.66%
Preterm	8	10.66%
IUGR	1	1.33%
LBW	6	8%
Neonatal Complications	3	4%
Neonatal Death	1	1.33%
Normal	48	64%

Pregnancy outcome in the study group



Adverse pregnancy outcome in patients with normal MSAFP includes

LBW	1	2.17%
Preterm	1	2.17%
Incomplete abortion	1	2.17%
Neonatal death	1	2.17%

Relation of MSAFP Value and Pregnancy out come

MSAFP	Adverse Pregnancy Outcome	Normal Pregnancy Outcome
Abnormal MSAFP	23	6
Normal MSAFP	4	42

29 patients had abnormal MSAFP values out of which 28 patients had elevated MSAFP with an adverse pregnancy outcome in 23 cases and 1 patient had a low MSAFP but she had a normal pregnancy outcome.46 patients had normal MSAFP with an adverse pregnancy outcome in 4 cases.

Sensitivity= 85.18%

Specificity = 87.5%

Positive predictive value =79.3%

Negative predictive value = 91.3%

Discussion

This study population consists of 75 pregnant high risk women between GA 15 to 22 weeks and were screened with MSAFP

MSAFP level observed in the study

MSAFP	Present Study (%)	Shanti Yadav et al (1993) (%)
Normal	61.33	24
Elevated	37.33	70
Low	1.33	6

MSAFP value in this study group was normal in 61.33% of patients, elevated in 37.33% and low in 1.33%

Percentage Detection of NTD

Study Group	Percentage Detection of NTD
Present study	100 %
Dipika Loghany	72.7%

In most studies ≥ 95% anencephaly, 75 to 90% of other open NTDs and 85% of all ventral wall defects are detected. (ACMG policy statement by Deborah and Driscoll 2004)

Early pregnancy loss and abnormal MSAFP

Study Group	MSAFP		
	Normal	Elevated	Low
Present Study			
Incomplete Abortion	50%	50%	Nil
Complete abortion	Nil	100%	Nil
Shanthi Yadav et al	4.5%	72.77%	22.7%
V.K Singh et al			
Incomplete Abortion	-	+	-
Missed Abortion	-	+	-

In early pregnancy loss the MSAFP levels are elevated in about 72.72%, low in 22.72% and normal value in 4.5% as reported by Shanti Yadav et al (1993).

Other studies which showed associations between early pregnancy loss and abnormal MSAFP include

1. Krause et al in their study of 77,149 showed a U shaped relation, with both low and high MSAFP values associated with increased incidence of early pregnancy loss.

- In MSAFP value < 0.25 MOM – a RR of early pregnancy loss of 15% is seen.
- In MSAFP value >2.5 MOM – a RR of early pregnancy loss of 12.5% is seen.

2. Vinita Das et al showed in her study that MSAFP level is significantly elevated (P < 0.001) in patients with threatened and inevitable abortion. On the other hand, MSAFP values were significantly low (P < 0.01) in patients with missed abortion.

Preterm deliveries and MSAFP

Study Group	MSAFP		
	Normal	Elevated	Low
Present study	12.5 %	87.5%	Nil
Shanti Yadav et al	Nil	100%	Nil

Other studies correlating abnormal MSAFP and preterm birth, include

1. Krause et al showed in his study showed a relative risk of 2.2 for preterm birth in MSAFP <0.25 and 4.8 for MSAFP ≥ 2.5
2. Simpson et al in his group of 650 patients without neural tube defects , noted that II trimester MSAFP is significantly elevated with PPROM and preterm birth

3. Cusick et al in a study of 383 patients found increased incidence of preterm births – 14.3 % , 15.6% and 20.3% with MSAFP ranges of 2 to 2.49 , 2.5 to 2.99 and >= 3 respectively
4. Waller and Cunningham studied records of 51,008 women. In those with elevated MSAFP , 24.3% had preterm births compared with 3.8% of women with decreased MSAFP

LBW and MSAFP

Study Group	MSAFP		
	Normal	Elevated	Low
Present study	16.66%	83.33%	Nil
Jyotsana Pandy et al	39.1%	60.9%	Nil

Other studies showing abnormal MSAFP and LBW include

1. Krause et al showed a relative risk of 1.4 for LBW with MSAFP < 0.25 MOM and 5.8 with values > 2.5 MOM
2. Cuckle et al showed that 94 pregnancies without NTD , but with MSAFP ≥ 3 resulted in LBW ($P < 0.001$)
3. Cusick et al in a study of 383 patients found increased incidence of LBW – 7.4%, 11.1% and 22.2% with MSAFP ranges of 2 to 2.49 MOM, 2.5 to 2.99 MOM, ≥ 3 MOM respectively
4. Simpson et al found significant elevation of MSAFP with LBW in a group of 650 patients
5. Milunsky et al in their study of 13,486 patients found a relative risk of 4 for patients with elevated MSAFP

Still birth and abnormal MSAFP

Study Group	MSAFP		
	Normal	Elevated	Low
Present study	Nil	100%	Nil
Dipika Loghany et al	16.66%	77.77%	1%

Other studies relating still birth and abnormal MSAFP include

1. Krause et al in their study showed a relative risk of 3.2 for MSAFP value <0.25 MOM and 0.9 for MSAFP > 2.5 MOM
2. Milunsky et al showed a RR of 3.3 for still birth in women with abnormal MSAFP.
3. Waller and Cunningham in their case control study showed that women with highest levels of serum alpha feto protein ≥ 3 MOM had a very high risk of fetal death – odds ratio of 104 and those with 2 – 2.9 MOM had a odds ratio of 2.4 than women with normal MSAFP

Neonatal complications and abnormal MSAFP

Study Group	MSAFP		
	Normal	Elevated	Low
Present study	Nil	100%	Nil
Shanthi Yadav et al	40%	60%	Nil

In the present study of 75 pregnant patients 4% had neonatal complications. Among the patients with neonatal complications 100% had elevated MSAFP compared to 60% in Shanti Yadav et al study

1. Milunsky et al in their study showed a relative risk of 3.6 for neonatal complications in case of abnormal MSAFP

Neonatal death and abnormal MSAFP

Study Group	MSAFP		
	Normal	Elevated	Low
Present study	Nil	100%	Nil
Jyotsana Pandey et al	16.66%	83.33%	Nil

In the present study 1 patient had neonatal death, but the mother had normal MSAFP.

Milunsky et al showed a relative risk of 15.9 for abnormal MSAFP.

Summary

1. The study group comprised of 75 high risk pregnant patients in gestational age 15 to 22 weeks and MSAFP was estimated in all patients
2. MSAFP values were elevated in 37.33% (> 2.5 MOM), normal in 61.33% and low in 1.33% (<0.25 MOM)
3. Ultra sonogram was done in patients with elevated MSAFP. In 3 cases congenital anomalies were found out of which 2 (75%) cases of neural tube defects and 1 (25%) case of omphalocele was found and the pregnancy was terminated. Other cases were followed till delivery.
4. MSAFP was elevated in 100% of cases with anomalies, fetal death, IUGR and neonatal complications.
5. Adverse pregnancy outcomes noted were
    - Anomalies – 4%
    - Early pregnancy loss – 4%
    - IUD – 2.66%
    - Preterm – 10.66%
    - IUGR – 1.33%
    - LBW (excluding preterm) – 8%

- Neonatal complications – 4%
- Neonatal death – 1.33%
- Normal outcome in 64% of cases

6. Strong associations were found between elevated MSAFP and the following adverse pregnancy outcomes
    - Anomalies – 100%
    - Preterm deliveries – 87.5%
    - Early pregnancy loss – 66.66%
    - LBW – 83.33%
    - IUD – 100%
    - Neonatal complications – 100%
    - IUGR – 100%
7. This study results clearly indicate the chance of adverse pregnancy outcome were increased in patients with elevated MSAFP
8. The sensitivity of the screening program in this study in 85.18%, specificity – 87.5%, positive predictive value – 79.3% and negative predictive value – 92.30%.

Conclusion

The conclusion arrived from the present study are

- This study indicates an association between adverse pregnancy outcome and neonatal complications with elevated MSAFP.
- A careful antenatal supervision is indicated in patients with elevated or low level of MSAFP.
- The screening procedure helps us to offer therapeutic termination to patient when there is an anomalous fetus.
- Elevated MSAFP level is associated with poor fetal outcome and should be considered as ominous prenatal finding.

Thus this screening procedure of MSAFP provide physician an important information and helps physician in identifying high risk patients and providing special care and specific investigations.

PROFORMA

Name : **Address :**

Age :

IP No :

Lab No :

Height :

Weight : **Phone No :**

Obstetric Code:

LMP		**EDD**		**GA**	

Dating Scan

DATE	CRL	BPD	FL	LIQUOR	GA

Menstrual H/O

Marital H/O

Previous Obstetric H/O

Term	
Pre-term	
Abortion/ IUD	
Associated anomalies	
H/O PIH, GDM, Hydramnios	

Medical H/O

Anemia, Hypertension	
Diabetes	
Epileptic on treatment	
Fever/Drug/Radiation in I trimester	
H/O Jaundice / Ovarian tumour	

Family H/O

H/O neural tube defects	
Other congenital anomalies	
Chromosomal disorder	

High risk factors (if any)

Investigations

Hb%		
Blood grouping/typing		
Blood sugar		
VDRL		
Urine	**Albumin**	
	Sugar	
	Deposits	

MSAFP Value

USG:

Pregnancy Outcome

Abortion /IUD

Pre-term

LBW

Still Birth

Term

Neonatal Period

Master Chart

Master Chart.xls

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ABBREVIATIONS

µg	Micro Gram
ACOG	American College of Obstetrics and Gynaecology
AFAFP	Amniotic Fluid Alpha Feto Protein
AFP	Alpha Feto Protein
Alb	Albumin
BOH	Bad Obstetric History
BPD	Biparietal Diameter
C	Celcius
cm	Centimeter
CPD	Cephalopelvic Disproportion
EPL	Early Pregnancy Loss
FL	Femur Length
GA	Gestational Age
gms	Grams
H/O	History of
Hb	Hemoglobin
hCG	Human Chorionic Gonadotropin
IDDM	Insulin Dependant Diabetes Mellitus
IUD	Intra Uterine Death
IUGR	Intra Uterine Growth Restriction
Kg	Kilogram
LBW	Low Birth Weight

LMP	Last Menstrual Period
LSCS	Lower Segment Caesarean Section
mg	Milligram
MOM	Multiples of Median
MSAFP	Maternal Serum Alpha Feto Protein
NC	Neo Natal Complications
ng	Nanogram
NTD	Neural Tube Defects
OPD	Out Patient Department
PAPPA	Pregnancy Associated Plasma Protein A
RR	Relative Risk
RSRM	Raja Sir Ramasami Mudaliar
uE_3	Unconjugated Estriol
USG	**Ultra sonogram**
VDRL	Venereal Disease Research Laboratory